

CLAIMS

1. A substantially pure preparation of a plasmin inhibitor characterised in that it is a single stage competitive inhibitor of plasmin.
2. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation constant for plasmin in the range of from $1 \times 10^{-8} \text{ M}^{-1}$ to $1 \times 10^{-10} \text{ M}^{-1}$.
3. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation constant for plasmin in the range of from $5 \times 10^{-8} \text{ M}^{-1}$ to $8 \times 10^{-9} \text{ M}^{-1}$.
4. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation constant for plasmin in the range of from $1 \times 10^{-9} \text{ M}^{-1}$ to $5 \times 10^{-9} \text{ M}^{-1}$.
5. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation rate constant for plasmin in the range of from $4 \times 10^{-5} \text{ sec}^{-1} \text{ M}^{-1}$ to $5 \times 10^{-7} \text{ sec}^{-1} \text{ M}^{-1}$.
6. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation rate constant for plasmin in the range of from $1 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$ to $1 \times 10^{-7} \text{ sec}^{-1} \text{ M}^{-1}$.
7. The plasmin inhibitor of claim 1 further characterised in that it has a dissociation rate constant for plasmin in the range of from $2 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$ to $9 \times 10^{-6} \text{ sec}^{-1} \text{ M}^{-1}$.
8. The plasmin inhibitor of claim 1 comprising a polypeptide selected from the group consisting of:
 - (a) Lys-Asp-Arg-Pro-Asp-Phe-Cys-Glu-Leu-Pro-Ala-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-Tyr-Asn-Pro-Asp-Glu-Lys-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Glu-Gly-Asn-Ala-Asn-Asn-Ph-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:2];

(b). Lys-Asp-Arg-Pro-Glu-Leu-Cys-Glu-Leu-Pro-Pro-Asp-Thr-Gly-Pro-Cys-
Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-Tyr-Asn-Pro-Asp-Glu-Gln-Lys-Cys-
Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-
Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:4];

5 (c). Lys-Asp-Arg-Pro-Asn-Phe-Cys-Lys-Leu-Pro-Ala-Glu-Thr-Gly-Arg-Cys-
Asn-Ala-Lys-Ile-Pro-Arg-Phe-Tyr-Tyr-Asn-Pro-Arg-Gln-His-Gln-Cys-
Ile-Glu-Phe-Leu-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-
Thr-Ile-Lys-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:6];

(d). Lys-Asp-His-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Glu-Thr-Gly-Ser-Cys-
10 Lys-Gly-Asn-Val-Pro-Arg-Phe-Tyr-Tyr-Asn-Ala-Asp-His-His-Gln-Cys-
Leu-Lys-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-
Thr-Ile-Glu-Glu-Gly-Lys-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:8];

(e). Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Leu-Pro-Asp-Thr-Gly-Ser-Cys-
Glu-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Thr-Arg-Asp-Arg-Glu-
15 Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Ile-
Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:10]; and

(f). Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Asp-Ile-Gly-Pro-Trp-
Asp-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Pro-Arg-Glu-His-Glu-Cys-
Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Lys-Gly-Asn-Ala-Asn-Asn-Phe-Asn-
20 Thr-Gln-Glu-Gln-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:12];

(g). a biologically-active fragment of any one of SEQ ID NO:2, 4, 6, 8, 10 and
12; and

(h). a variant or derivative of any of the foregoing polypeptides or fragments
thereof.

9. The plasmin inhibitor of claim 8 wherein said variant has the general formula:
KDZPZYCZLBBZBGXCZXXXBXFAYXBZZZCBZFBYGGCXBNANNF
XTXEECESTCAA (I), wherein: -

25

Art B9

X is any amino acid;
Y is a hydrophobic amino acid;
A is an aromatic amino acid;
Z is K, R, H, D, E, Q or N; and
5 B is a neutral amino acid, or P, A, G, S, T, V or L.

10. The plasmin inhibitor of claim 9, wherein the Z at position 3 is H or R.
11. The plasmin inhibitor of claim 9, wherein the Z at position 5 is K, N, E or D.
12. The plasmin inhibitor of claim 9, wherein the Y at position 6 is F or L.
13. The plasmin inhibitor of claim 9, wherein the Z at position 8 is E or K.
- 10 14. The plasmin inhibitor of claim 9, wherein the B at position 10 is P or L.
15. The plasmin inhibitor of claim 9, wherein the B at position 11 is P or A.
16. The plasmin inhibitor of claim 9, wherein the Z at position 12 is E or D.
17. The plasmin inhibitor of claim 9, wherein the B at position 13 is T or I.
18. The plasmin inhibitor of claim 9, wherein the X at position 15 is P, S or R.
- 15 19. The plasmin inhibitor of claim 9, wherein the Z at position 17 is K, N, E, D or R.
20. The plasmin inhibitor of claim 9, wherein the X at position 18 is D, G, A or V.
21. The plasmin inhibitor of claim 9, wherein the X at position 19 is F, N, K or R.
22. The plasmin inhibitor of claim 9, wherein the X at position 20 is T, P, F or I.
- 20 23. The plasmin inhibitor of claim 9, wherein the B at position 21 is G, V or P.
24. The plasmin inhibitor of claim 9, wherein the X at position 22 is A, S or R.
25. The plasmin inhibitor of claim 9, wherein the A at position 24 is Y or H.
26. The plasmin inhibitor of claim 9, wherein the X at position 26 is S or N.
27. The plasmin inhibitor of claim 9, wherein the B at position 27 is P, A or T.
- 25 28. The plasmin inhibitor of claim 9, wherein the Z at position 28 may be D or R.
29. The plasmin inhibitor of claim 9, wherein the Z at position 29 is E, D, H or Q.
30. The plasmin inhibitor of claim 9, wherein the Z at position 30 is H, K, R or Q.

31. The plasmin inhibitor of claim 9, wherein the Z at position 31 is K, Q or E.
32. The plasmin inhibitor of claim 9, wherein the B at position 33 is L or I.
33. The plasmin inhibitor of claim 9, wherein the Z at position 34 is E or K.
34. The plasmin inhibitor of claim 9, wherein the B at position 36 is L or I.
- 5 35. The plasmin inhibitor of claim 9, wherein the X at position 41 is E, G or K.
36. The plasmin inhibitor of claim 9, wherein the B at position 42 is C or G.
37. The plasmin inhibitor of claim 9, wherein the X at position 48 is K, N or I.
38. The plasmin inhibitor of claim 9, wherein the X at position 50 is K, Q or I.
39. The plasmin inhibitor of claim 8, wherein the polypeptide comprises a leader
10 peptide comprising the sequence:- Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-
Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser [SEQ ID
NO:14], or a biologically-active fragment thereof, or variant or derivative of
these.
40. The plasmin inhibitor of claim 39, wherein the polypeptide is selected from
15 the group consisting of:-
 - (a) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Asp-Phe-Cys-Glu-
Leu-Pro-Ala-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-
Tyr-Asn-Pro-Asp-Glu-Lys-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-
20 Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-
Cys-Ala-Ala [SEQ ID NO:16];
 - (b) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-
Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Glu-Leu-Cys-Glu-
Leu-Pro-Pro-Asp-Thr-Gly-Pro-Cys-Arg-Val-Arg-Phe-Pro-Ser-Phe-Tyr-
25 Tyr-Asn-Pro-Asp-Glu-Gln-Lys-Cys-Leu-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-
Glu-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-
Cys-Ala-Ala [SEQ ID NO:18];

(c) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Asn-Phe-Cys-Lys-Leu-Pro-Ala-Glu-Thr-Gly-Arg-Cys-Asn-Ala-Lys-Ile-Pro-Arg-Phe-Tyr-Tyr-Asn-Pro-Arg-Gln-His-Gln-Cys-Ile-Glu-Phe-Leu-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Lys-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:20];

(d) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-His-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Glu-Thr-Gly-Ser-Cys-Lys-Gly-Asn-Val-Pro-Arg-Phe-Tyr-Tyr-Asn-Ala-Asp-His-His-Gln-Cys-Leu-Lys-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Lys-Thr-Ile-Glu-Glu-Gly-Lys-Ser-Thr-Cys-Ala-Ala [SEQ ID NO:22];

(e) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Leu-Pro-Asp-Thr-Gly-Ser-Cys-Glu-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Thr-Arg-Asp-Arg-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Gly-Gly-Asn-Ala-Asn-Asn-Phe-Ile-Thr-Lys-Glu-Glu-Cys-Glu-Ser-Thr-Cys-Ala-Ala; [SEQ ID NO:24]; and

(f) Met-Ser-Ser-Gly-Gly-Leu-Leu-Leu-Leu-Gly-Leu-Leu-Thr-Leu-Trp-Glu-Val-Leu-Thr-Pro-Val-Ser-Ser-Lys-Asp-Arg-Pro-Lys-Phe-Cys-Glu-Leu-Pro-Ala-Asp-Ile-Gly-Pro-Trp-Asp-Asp-Phe-Thr-Gly-Ala-Phe-His-Tyr-Ser-Pro-Arg-Glu-His-Glu-Cys-Ile-Glu-Phe-Ile-Tyr-Gly-Gly-Cys-Lys-Gly-Asn-Ala-Asn-Asn-Phe-Asn-Thr-Gln-Glu-Gln-Cys-Glu-Ser-Thr-Cys-Ala-Ala; [SEQ ID NO:26].

25 41. An isolated polynucleotide encoding the polypeptide of claim 8.

42. An isolated polynucleotide selected from the group consisting of:-

(a) AAGGACCGTCCGGATTTCTGTGAAGTGCCTGCTGACACCGGACC

ATGTAGAGTCAGATTCCCATCCTCTACTACAACCCAGATGAAAAA
AAAGTCTAGAGTTATTTATGGTGGATGCGAAGGGAATGCTAA
CAATTTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCCT
GA [SEQ ID NO:1];

5 (b) AAGGACCGTCCAGAGTTGTGTGAAC TGCTCCTGACACCGGACC
ATGTAGAGTCAGATTCCCATCCTCTACTACAACCCAGATGAACA
AAAATGCCTAGAGTTATTTATGGTGGATGCGAAGGGAATGCTA
ACAATTTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCC
TGA [SEQ ID NO:3];

10 (c) AAGGACCGTCCAAATTCTGTAAACTGCCTGCTGAAACCGGACG
ATGTAATGCCAAAATCCCACGCTTCTACTACAACCCACGTCAAC
ATCAATGCATAGAGTTCTATGGTGGATGCGGAGGGAATGCT
AACAAATTAAAGACCATAAGGAATGCGAAAGCACCTGTGCTGC
ATGA [SEQ ID NO:5];

15 (d) AAGGACCATCCAAAATTCTGTGAAC TCCCTGCTGAAACCGGATC
ATGTAAAGGCAACGTCCCACGCTTCTACTACAACGCAGATCATC
ATCAATGCCTAAAATTATTTATGGTGGATGTGGAGGGAATGCTA
ACAATTTAAGACCATAAGGAAGGCAAAAGCACCTGTGCTGCC
TGA [SEQ ID NO:7];

20 (e) AAGGACCGTCCAAAATTCTGTGAAC TGCTCCTGACACCGGATC
ATGTGAAGACTTACCGGAGCCTCCACTACAGCACACGTGATC
GTGAATGCATAGAGTTATTTATGGTGGATGCGGAGGGAATGCT
AACAAATTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGC
CTGA [SEQ ID NO:9];

25 (f) AAGGACCGTCCAAAGTTCTGTGAAC TGCTGCTGACATCGGACC
ATGGGATGACTTACCGGAGCCTCCACTACAGCCCACGTGAAC
ATGAATGCATAGAGTTATTTATGGTGGATGCAAAGGGAATGCT

AACAACTTAATACCAAGAGCAATGCGAAAGCACCTGTGCTGC
CTGA [SEQ ID NO:11];

(g) a polynucleotide fragment of any one of SEQ ID NOS 1, 3, 5, 7, 9 and 11,
wherein said polynucleotide fragment encodes a biologically-active
fragment of any one of SEQ ID NO:2, 4, 6, 8, 10 and 12; and

5 (h) a polynucleotide homologue of any of the foregoing sequences.

43. The polynucleotide of claim 42 further comprising a nucleotide sequence
encoding a leader peptide.

44. The polynucleotide of claim 43, wherein the nucleotide sequence comprises
10 the sequence:-

ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCTG
GGAGGTGCTGACCCCCGTCTCCAGC [SEQ ID NO:13] or a biologically
active fragment thereof, or a polynucleotide homologue of these.

45. The polynucleotide of claim 43, wherein said polynucleotide is selected from
15 the group consisting of:-

(a) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCGGATTTC
TGTGAAC TG C CTG CTG AC ACC GG ACC AT GT AG AG TC AG ATT CCC
ATCCTTCTACTACAACCCAGATGAAAAAAAGTGCCTAGAGTTAT
20 TTATGGTGGATGCGAAGGGAATGCTAACAAATTATCACCAAAG
AGGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:15];

(b) ATGTCTTCTGGAGGTCTTCTTCTCCTGCTGGGACTCCTCACCCCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAGAGTTG
TGTGAAC TG C CTG CTG AC ACC GG ACC AT GT AG AG TC AG ATT CCC
TCCTTCTACTACAACCCAGATGAACAAAAATGCCTAGAGTTATT
25 TATGGTGGATGCGAAGGGAATGCTAACAAATTATCACCAAAGA
GGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:17];

(c) ATGTCTTCTGGAGGTCTTCTCCTGCTGGACTCCTCACCCCTCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAATTTC
TGTAAACTGCCTGCTGAAACCGGACGATGTAATGCCAAAATCCC
ACGCTTCTACTACAACCCACGTCAACATCAATGCATAGAGTTCT
5 CTATGGTGGATCGGGAGGGAAATGCTAACAAATTAAAGACCATTA
AGGAATGCGAAAGCACCTGTGCTGCATGA [SEQ ID NO:19];

(d) ATGTCTTCTGGAGGTCTTCTCCTGCTGGACTCCTCACCCCTCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCATCCAAAATTTC
TGTGAACCTCCCTGCTGAAACCGGATCATGTAAGGCAACGTCCC
10 ACGCTTCTACTACAACGCAGATCATCATCAATGCCTAAAATTAT
TTATGGTGGATGTGGAGGGAAATGCTAACAAATTAAAGACCATAG
AGGAAGGCAAAAGCACCTGTGCTGCCTGA [SEQ ID NO:21];

(e) ATGTCTTCTGGAGGTCTTCTCCTGCTGGACTCCTCACCCCTCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAATTTC
15 TGTGAACTGCTCCTGACACCGGATCATGTGAAGACTTTACCGGA
GCCTTCCACTACAGCACACGTGATCGTGAATGCATAGAGTTATT
TATGGTGGATGCGGAGGGAAATGCTAACAAATTATCACCAAAGA
GGAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:23];

(f) ATGTCTTCTGGAGGTCTTCTCCTGCTGGACTCCTCACCCCTCT
GGGAGGTGCTGACCCCCGTCTCCAGCAAGGACCGTCCAAAGTTTC
20 TGTGAACTGCTGCTGACATCGGACCATGGGATGACTTTACCGG
AGCCTTCCACTACAGCCCACGTGAACATGAATGCATAGAGTTATT
TTATGGTGGATGCAAAGGGAAATGCTAACAACTTTAATACCCAAG
AGCAATGCGAAAGCACCTGTGCTGCCTGA [SEQ ID NO:25]; and

25 (g) GGAGCTTCATCATGTCTTCTGGAGGTCTTCTCCTGCTGGGAC
TCCTCACCCCTGGGAGGTGCTGACCCCCGTCTCCAGCAAGGACC
GTCCAGAGTTGTGTGAACGCCTCCTGACACCGGACCATGTAGA

GTCAGATCCCCATCCTTCTACTACAACCCAGATGAACAAAAATG
CCTAGAGTTTATTATGGTGGATGCGAAGGGAATGCTAACCAATT
TTATCACCAAAGAGGAATGCGAAAGCACCTGTGCTGCCTGAATG
AGGAGACCCCTCCTGGATTGGATCGACAGTTCCAACTTGACCCAA
5 AGACCCCTGCTTCTGCCCTGGACCACCCCTGGACACCCTCCCCAA
ACCCCACCCCTGGACTAATTCTTCTGCAATAAGCTTGTT
TCCAGCT [SEQ ID NO:43]

46. A pharmaceutical composition for alleviating blood loss in a patient, said composition comprising the polypeptide of claim 8 and a pharmaceutically acceptable carrier.

10 47. A method for alleviating blood loss comprising the step of administering to a patient in need of such treatment a therapeutically effective dosage of the polypeptide of claim 8 in combination with a pharmaceutically acceptable carrier.

15 48. An anti-tumour agent comprising the polypeptide of claim 8 conjugated with an anti-fibrin antibody.